THE ORIGIN OF YOLK IN THE OVA OF AN ENDOPARASITIC COPEPOD.

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(Plate iii.).

In January, 1905, Professor J. P. Hill presented me with material for the study of the morphology and development of an endoparasitic copepod infesting *Ptychodera australiensis* Hill. The parasite was found to be new, and was subsequently described under the name of *Ubius hilli* Kestv., in 1913(3). The following observations were made on the developing, ultimate oögonium, and primary oöcyte at the time *U. hilli* was studied (1908), and were submitted to Prof. Hill for criticism. At his suggestion, this paper was withheld from publication because, at that time, the material on which the observations were made was all stained in one way. Since then, I have obtained fresh material, which was fixed in (*a*) 5% formaldehyde in seawater; and (*b*) Müller's bichromate-solution. Specimens, after cutting, were stained with (1) Delafield's hæmatoxylin, (2) in Heidenhain's hæmatoxylin, (3) Mayer's carm-alum, (4) Flemming's method for karyokinetic figures.

This staining was done over three years ago, but press of other work has, till now, prevenced me from examining the results. Recently, I have found time to go over these sections, and I find that I have nothing to add to the original paper. I am satisfied, that the observations recorded truly represent processes taking place in the developing cell, and are not artifacts, for I find the cells to present these appearances, not only in different specimens similarly stained, but also in the same and in different specimens dissimilarly stained. Were I situated in a city near a library, I doubt not I would find many reasons for altering

that portion of the following paper which deals with the literature. This paper was written in 1908, and since then I have had no opportunity of keeping abreast of the cognate current literature, and, at present, I am beyond the reach of a library. I believe, however, that even though this contribution is thus nine years old at date of printing, it is deserving of publication.

The ovarian epithelium in the young female is composed of fairly regular, cubical oögonia.* The continued division of these gives rise to the mass of ultimate oögonia which fills not only the lumen of the ovary, but also the anterior portion of that of the oviduct. It is while lying free in the lumen, in this mass, that the ultimate oögonium advances so far towards maturity, that it may thereafter be regarded as the primary oöcyte.

When first shed into the lumen of the gland, the ultimate oögonium is a small, rounded, hyaline cell. Its comparatively large nucleus may contain only one karyosome, or it may contain two, three, or four of approximately equal size. The size of the nucleus is defined by the nuclear membrane alone; that is to say, the nucleoplasm is hyaline, and takes no stain, nor is it differentiated from the cytoplasm by the presence of a discernible chromatin-reticulum (Fig.1). In those cases where there is only one karyosome, I am unable to find that this *one* differs from the three or four in other cases, nor among these is there any difference *inter se*.

The cell now enlarges. The nucleus, increasing in size at a greater rate than the cell, comes ultimately to fill nearly the whole cell.

When this process has reached the stage depicted in Fig.2, a chromatin-reticulum is well established, and the karyosomes have increased in number. In short, the period is characterised by an increase of chromatin.

As soon as this increase has reached its maximum, the reticulum becomes broken down, till, as depicted in Fig.3, in place of a network, there is present a great number of fine granules of

* The description is of material stained with hæmatoxylin without any counter stain.

chromatin, and the karyosomes lie free in the nucleoplasm. The period is characterised by the disintegration of the chromatin-reticulum formed during the last period.

The fine granules of chromatin next begin to increase in size, and lose in depth of staining, until, instead of being opaque black points, they become semi-translucent, purple spherules. The karyosomes meanwhile remain unchanged (Fig.4). This phenomenon, I regard as the formation of the first yolk-granules. During succeeding stages, they continue to increase in size.

The karyosomes very soon exhibit signs of activity. Each of them, from a solid sphere of chromatin, becomes converted into a small, spongiform mass (Fig.5), probably due to the formation of vacuoles within them. What this activity, which characterises the period, means, I am quite unable to say, but I do not think that it can affect the deductions made later.

Meanwhile, the nucleus has so increased in size, that it is now surrounded by a mere envelope of cytoplasm, the presence of the nuclear membrane being evidenced more by the definite outline of the nucleus, than by the actual visibility of the membrane.

The next period is characterised by the formation of a new and much smaller nucleus within the old. This takes place in three steps.

Around one, or it may be two or three, coalescent, spongiform karyosomes, there becomes recognisable an area of plasm devoid of the spherules, which are scattered plentifully throughout the rest of the nucleus, and this area of plasm takes a faint purple stain (Fig.6).

This purple-staining globule of plasm, with its contained karyosome or karyosomes, by this time quite coalesced, is next enclosed in a distinct membrane (Fig.7).

The karyosome once more assumes a solid spherical form, the surrounding plasm still staining purple.

This area henceforward constitutes the nucleus of the cell; it is shown in Fig.8, which is a section of a mature, primary oöcyte.

Meanwhile the remaining karyosomes, scattered throughout the old nuclear area, have also shrunken to their previous size and

shape, and again become solid; during this time, none of them were surrounded by a clear area of plasm staining purple.

The old nuclear membrane is apparently still present, in some cases, until a much later period; but, in others, it seems to have broken down at the time when the new nucleus first develops its membrane.

Up to this time, the ultimate oögonium has lain free in the lumen of the gland or oviduct; it now becomes attached to the wall of the latter, either to one cell by a foot, as in Fig.9, or, in the absence of the foot, to several cells.

Growth in size appears now to take place very rapidly, and there is a great increase in the quantity of yolk-spherules. No changes are observable in the new nucleus.

Concurrently with the increase in size of the cell and quantity of yolk, the karyosomes left free in the old nuclear area appear to be dissolved without showing any further signs of activity. When the cell, now to be regarded as a primary oöcyte, has reached its full size, it is once more set free into the lumen of the oviduct.

There is present in the mature primary oöcyte only one set of spherules, variable in size, certainly, but differing in no other way from one another. All are yolk-spherules or none are yolk-spherules. The spherules which were formed by increase in size of the chromatin-granules are, therefore, similar to, and may be regarded as being the same as, those formed later.

After these observations had been made, and the conclusions given below had been deduced from them, I sought, in the publications of previous workers, for comparable observations and deductions. I cannot better give the results of my examination of literature than by the following quotation from a paper by Caroline McGill(5), who, on p.219 of the paper quoted, expresses the opinion that "it seems probable that chromatin may have something to do with yolk-formation."

Will(7) thinks that the larger nucleoli of the amphibian germinal vesicle pass out into the cytoplasm, and there become yolk-nuclei. MacCallum(4) concludes that, in the ova of Am-

phibia, the peripheral nucleoli generate a substance which diffuses first into the nucleus, and from there into the cytoplasm; finally, it combines with the cytoplasm to form yolk. Henneguy (2) believes that the corpuscles of Balbiani in vertebrates are either parts of the nucleolus, or the entire nucleolus, which pass through the nuclear wall into the cytoplasm. Montgomery (6), in *Pisicola*, describes the nucleus as contracting in volume, and, in so doing, discharging all except one of its nucleoli into the cytoplasm. Goldschmidt (1), in active gland-cells and in muscle-cells of *Ascaris*, has described a cytoplasmic chromatin, which, instead of being gathered into irregular masses as in the Nissl bodies of nerve-cells, is arranged in fibres or coarse reticula. In most instances, however, this chromatin, which he calls 'Chromidialapparat,' is not derived from nucleolar material, but represents nuclear chromatin which has made its way into the cytoplasm. In fact, in many cases, the chromatin-fibres of the cytoplasm extend directly through the nuclear membrane, and are continuous with the chromatic reticulum of the nucleus. Since the chromatic apparatus is more highly developed in active than in resting cells, Goldschmidt concludes that it must function in the metabolism of the cytoplasm.

My own deductions may now be put very briefly. They are:—

1. The yolk-granules are formed by the combination of a cytoplasmic constituent with chromatin.

2. The first yolk-granules are formed within the nucleus.

3. The formation of the new nucleus is a pseudo-contraction of the overladen, old nucleus.

4. This pseudo-contraction leads to the shedding of some of the karyosomes, which are henceforward to be regarded as yolk-nuclei.

5. These yolk-nuclei are stores of chromatin, which are to continue the functional activity of the nucleus of the growing primary oöcyte, that is to say, they are to supply chromatin for that combination which is yolk-formation.

6. The ultimate oögonium is nourished by endosmosis, the primary oöcyte by the epithelium of the oviduct. In both cases, the all-important substance received is the cytoplasmic constituent

which enters into yolk-formation. Chromatin-constituents, however, must be derived from without in the earliest stages.

LITERATURE.

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EXPLANATION OF PLATE III.

Explanatory letters.—K., karyosomes—Nu′., the new nucleus in process of formation—Nu″., the new nucleus nearly formed—Nu. memb′., the nuclear membrane of the old nucleus—Nu. memb″., the nuclear membrane of the new nucleus.

Fig. 1.—The ultimate oögonium when first detached from the wall of the ovary.

Figs. 2, 3, 4, 5.—Stages in the growth of the ultimate oögonium.

Figs. 6, 7.—Stages in the formation of the new nucleus.

Figs. 8, 9.—Sections of mature primary oöcytes.